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Title: HYDRAZINE-BASED AND C BASED BIFUNCTIONAL
CROSSLINKING REAGENTS
Applicant: David A. Schwartz
Serial No. To be assigned
Our Docket No.: 37154-753

# FIGURE 1

$$\begin{matrix} R \\ N \end{matrix} \begin{matrix} NH_2 \\ H \end{matrix}$$

$$\begin{array}{c|c} & O \\ R & & NH_2 \\ \hline & & H & H \end{array}$$

$$\begin{matrix} & & H & H \\ I & & I \\ N & & N \\ I & & N \\ I & & O \end{matrix} \\ \begin{matrix} H & & H \\ I & & \\ N & & \\ NH_2 \end{matrix}$$

hydrazine

semicarbazide

$$R \underset{O}{\overset{H}{\underset{|}{\bigvee}}} NH_2$$

hydrazide

Sund Verre Terre Series Series

thiosemicarbazide

thiocarbazide

$$\begin{array}{c|c} O & H & H \\ \hline R & N & N & N \\ \downarrow & \downarrow & N \\ H & H & O \end{array}$$

$$\begin{array}{c} O \\ R \\ O \\ H \end{array}$$

carbonic acid dihydrazine

hydrazine carboxylate

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protein A 
$$NH_2$$
  $NH_2$   $NH_2$   $NH_3$   $NH_4$   $NH_4$   $NH_4$   $NH_4$   $NH_4$   $NH_5$   $NH_4$   $NH_5$   $NH_4$   $NH_5$   $NH_4$   $NH_5$   $NH_4$   $NH_5$   $NH_5$   $NH_5$   $NH_6$   $NH_6$ 

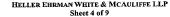
protein B 
$$NH_2$$
  $NH_2$   $NH_2$   $NH_2$   $NH_3$   $NH_4$   $NH_4$ 

$$\mathbf{H} + \mathbf{A} \xrightarrow{\text{pH 4.7 or 7.4}} \mathbf{protein A} \xrightarrow{\mathbf{H}} \mathbf{N} \xrightarrow{\mathbf{N}} \mathbf{N} \xrightarrow{\mathbf{N}} \mathbf{N}$$



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### FIGURE 5

#### Preparation of 5'-aromatic alehyde-modified oligonucleotide:

Preparation of 5'-heteroaromatic hydrazine-modified oligonucleotide:

oli gonucleotide – 
$$(CH_2)_6NH_2$$

$$\frac{PBS, pH 7.4}{2) 0.1 \text{ M acetate, pH 4.7}} \text{ oli gonucleotide-5'-}(CH_2)_6NH$$

$$NH_2$$

Conjugation of hydrazino-modified oligonucleotide to aldehyde-modified oligonucleotide

oligonucleotide-5'-
$$(CH_2)_6NH$$
 +  $H_2N$  NH $(CH_2)_6$ -5'-oligonucleotide oligonucleotide oligonucleotide-5'- $(CH_2)_6NH$  ONH $(CH_2)_6$ -5'-oligonucleotide oligonucleotide oligonucleotide-5'- $(CH_2)_6NH$  NH $(CH_2)_6$ -5'-oligonucleotide

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## FIGURE 6

phosgene, Et3N, THF

$$OCN$$
 $OCN$ 
 $OCN$ 

t-butyl carbazate, THF

1) HCl/dioxane

2) acetone, MeOH

$$\begin{array}{c|c}
 & O \\
 & N \\$$

NHS, DCC, THF

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$$\begin{array}{c} \text{OEt} \\ \text{EtO} \\ \text{Si} \\ \text{NH}_2 \end{array} + \begin{array}{c} \text{SuO} \\ \\ \text{N} \\ \text{N} \\ \text{N} \end{array}$$

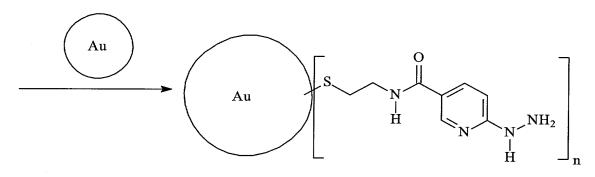


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$$H_2N$$
 $S$ 
 $NH_2$ 
 $NH_2$ 





## FIGURE 9

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